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Original article

Ventricular arrhythmia and torsade de pointe: Dose limiting toxicities of the MDR-modulator S9788 in a phase I trial

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Summary

Background: S9788 is a triazineaminopiperidine derivative capable of reversing multidrug resistance (MDR) *in vitro*. In preclinical models S9788 was several fold more potent MDR inhibitor than verapamil or cyclosporine. At P-glycoprotein (Pgp) blocking concentrations, S9788 appeared to have only very little toxicity.

Patients and methods: In a two step phase I trial we treated 39 patients with refractory cancer with S9788 and bolus doxorubicin. The steps differed mainly in the S9788 infusion duration; in the first part 23 patients received the MDR-reversing drug S9788 over 30 minutes, in the second step of the study 16 patients were administered S9788 over 150 minutes. The doses of S9788 were escalated in cohorts of three patients up to a dose level (DL) of 96 mg/m^2 on the 30 minutes infusion, and to 144 mg/m^2 on the 150 minutes infusion. The pharmacokinetics of S9788 were determined.

Results: With the 30-minute infusion schedule symptomatic cardiac arrhythmia were found to be dose limiting. In all patients at the highest DL transient cardiac repolarization prolongation with a long QT-interval on ECG was demonstrated. With the 150-minute administration schedule, S9788 could be escalated up to 144 mg/m^2 without subjective toxicity. However, transient QT prolongation was present in all patients. A third degree AV-block and a QT increase of about 40% occurred at the highest DL. Asymptomatic torsade de pointe (DL 96 mg/m^2) was demonstrated on Holter recording in one patient. Theses repolarization disturbances with QT increase were considered dose limiting toxicity and the trial was closed. No arrhythmia related death was noted. Pharmacokinetics were similar with both infusion schedules with a mean alpha half life of 11.3 and 13.2 minutes, for the 30-minute and 150-minute infusion, and a terminal half life of 13.5 and 15 hours, respectively. QTc prolongation duration appeared to be dose-dependent.

Conclusions: With the tested infusion schedules, cardiac toxicity, in particular AV-blocks and QT prolongation, leading to ventricular arrhythmia and torsade de pointe, are the dose limiting toxicities of S9788. Our experience together with the observation of asymptomatic torsade de pointe in two other phase I trials of S9788 infused over six hours precluded the further clinical development of S9788.

Key words: arrhythmia, long QT, MDR, P-glycoprotein, S9788, torsade de pointe, triazineaminopiperidine

Introduction

Tumor cell resistance to chemotherapy is a major cause of treatment failure. While some tumors are intrinsically resistant to chemotherapy, in others, resistance is an acquired phenomenon developing during chemotherapy [1, 2]. Multidrug resistance (MDR) frequently develops after initial successful treatment. Recurrent cancer then also exhibits resistance to structurally different drugs, to which it was never exposed. Resistance to multiple drugs may develop through a common molecular mechanism. MDR is frequently observed after treatment with doxorubicin and other anthracyclines, vinca alkaloids and epipodophyllotoxins [3]. MDR has also been reported for paclitaxel, mitomycin-C and trimetrexate. MDR has been recognized to be associated with increased expression of P-glycoprotein (Pgp) [4], a transmembrane protein which functions as an active efflux pump for cytotoxic agents [2, 5]. Pgp can be found in normal kidney, liver, colon and lung tissue, as well as in the bone marrow. *In vitro*, MDR overexpression can be reversed pharmacologically, e.g., by cyclosporine and its analogue PSC 833, verapamil, tamoxifen and other structurally not related drugs [5–11]. The mechanism of this interaction is not fully understood. *In vivo*, sufficient concentrations of many of these drugs cannot be achieved without substantial toxicity [12–14], and the development of new specific MDR-modulating drugs is warranted [15]. The SDZ compound PSC 833, a derivative of cyclosporine D without the immunosuppressive and nephrotoxic properties of its parent compound is currently undergoing clinical testing [16–18].

Another such a novel agent capable of reversing MDR is S9788. This triazineaminopiperidine derivative has no structural similarities to other known MDR-modulating agents. *In vitro*, S9788 has been shown to

effectively reverse MDR-dependent resistance to doxorubicin, vincristine and a variety of cytotoxic drugs [19, 20]. At a concentration of 5 μM it was 2.5–317 times more potent than verapamil in reversing MDR [21–23]. Efficacy of S9788 in reversing anthracycline resistance was also shown *in vivo* in animal models [24]. In a murine leukemia model resistant to anthracyclines, intraperitoneal injection of S9788 30 minutes before administration of doxorubicin resulted in significant antitumor activity [25]. Similarly, this effect could also be demonstrated with vincristine with even greater antitumor activity enhancement in the same model. The exact mechanism of action of S9788 remains unclear, it is believed to bind to Pgp and to alter intracellular drug distribution [26].

Based on these observations phase I studies were initiated using S9788 in different infusion schedules in association with doxorubicin-chemotherapy [27, 28]. In a two-step phase I study we evaluated toxicity and tolerability of S9788, infused alone, and in association with doxorubicin over 30 minutes and 150 minutes, respectively.

Patients and methods

Patient selection

Patients with advanced malignancies unresponsive to standard treatment and for which no other established therapy exists, and with documented progression during the preceding two months were eligible. Inclusion criteria included a life expectancy of ≥ 12 weeks, an interval since last chemotherapy or radiation ≥ 4 weeks, and discontinuation of any hormono- or immunotherapy for more than two weeks. Prior doxorubicin exposure was allowed, providing the cumulative dose did not exceed 300 mg/m^2. Other eligibility criteria were age 18–75 years, ECOG PS ≤ 2, adequate hematological (WBC $\geq 4.0 \times 10^9$/l, hemoglobin ≥ 10.0 g/dl, platelets $\geq 100 \times 109$/l), hepatic (ASAT, ALAT, alkaline phosphatase $\leq 2.5 \times$ upper limit of normal, bilirubin $\leq 1.25 \times$ upper normal limit), and renal parameters (creatinine $\leq 1.25 \times$ upper normal limit, creatinine clearance ≥ 60 ml/minute). Concomitant treatment with other potentially MDR modulating drugs (e.g., verapamil, cyclosporine) was not allowed. All patients gave written informed consent and the trial was approved by the participating institutions ethics committees.

Study design

In a first step (Table 1) patients were treated with S9788 as a 30-minute infusion. In order to evaluate the intrinsic toxicity of the MDR-reversant S9788 was administered alone on day 1 and 8 To better compare the tolerance to doxorubicin with and without the MDR reversant, the patients received a total dose of 20 mg of doxorubicin alone on day 15, while doxorubicin was administered immediately after completion of the S9788 infusion over 30 minutes on day 22. Then, combined treatment was repeated weekly for a minimum of seven weeks, or until severe toxicity or tumor progression. Because modulation of normal tissue Pgp by S9788 led to increased toxicity of doxorubicin in animal models while activity was retained with 50% of the cytotoxic drug dose, a low total dose of doxorubicin was chosen for its combination with S9788 in humans.

In a second step due to the observation of severe toxicity possibly related to the concentration peak with the 30-minute schedule, we evaluated a protracted infusion of S9788 over 150 minutes (DL 8–13).

Table 1 Dose levels and number of infusions.

Dose level number (mg/m^2)	*n*	Number of infusions		
		S9788	S9788 + doxo	Total
30-min infusion S9788				
1 (8)	1	2	3	5
2 (16)	4	8	24	32
3 (26)	3	6	36	42
4 (40)	3	6	22	28
5 (56)	3	6	16	22
6 (72)	3	6	14	20
7 (96)	6	12	23	35
Total 30-min infusion	23	46	123	183
150-min infusion S9788				
8 (72)	3	3	17	20
9 (96)	3	3	13	16
10 (80)	2	2	4	6
11 (104)	3	3	21	24
12 (120)	3	3	11	14
13 (144)	2	2	5	7
Total 150-min infusion	16	16	71	87

S9788 was administered alone on day 1, and with doxorubicin on day 8. The doxorubicin was given i.v. bolus over five to 10 minutes, starting half an hour after the beginning of the S9788 infusion. This treatment was again repeated weekly for seven weeks, or until severe toxicity or tumor progression.

The initial dose of S9788 for the 30-minute infusion was chosen based on 1/10 of the mouse LD_{10}. Subsequent dose escalation followed the modified Fibonacci-scheme (Table 1). Seven dose levels were investigated from 8 mg/m^2 up to 96 mg/m^2. The administration of S9788 as a continuous infusion over 150 minutes used the previously defined acceptable dose of the 30-minute infusion schedule (72 mg/m^2) as the starting dose. Due to cardiac toxicity at the subsequent dose level, an additional intermediate dose level of 80 mg/m^2 was introduced before again escalating the dose of S9788 up to 144 mg/m^2.

S9788 was supplied by Servier International Research Institute (Courbevoie, France), as a dimethane sulphonate salt solution in 10 ml vials containing 100 mg S9788. This solution was further diluted in 5% glucose for intravenous infusion.

Pretreatment and follow-up studies

At baseline, all patients had a complete physical exam, ECG, cardiac ejection fraction measured by echocardiography or MUGA-scan, CBC, kidney and liver function tests and adequate imaging of evaluable tumor. Physical exam and CBC were repeated weekly, ECG and blood chemistry every other week and cardiac ejection fraction was repeated after administration of four doses of doxorubicin. Patients were evaluated for toxicity weekly and toxicity were graded using the NCI common toxicity criteria. Tumor response was evaluated every six weeks. From dose level 6 on, when cardiac arrhythmias were recognized as a potentially severe toxicity, systematic cardiac monitoring was introduced consisting of an ECG performed before each S9788 infusion and by Holter recording starting approximately 15 minutes before until about half an hour after each S9788 infusion. In the second part of the study (150-minute infusion) all patients had peri-infusional Holter monitoring starting half an hour before the S9788 infusion and lasting until at least 30 minutes after the infusion end. QT-interval was measured and corrected for the heart rate (QTc) according to the Bazett formula: QTc (msec) = QT (msec) $\times \sqrt{RR}$ }, and a QTc of > 440 msec. was considered pathological.

Pharmacokinetic studies were performed on day 1 and day 8 during the first phase of the study evalutating the 30-minute infusion schedule

Table 2. Patient characteristics.

	Infusion duration	
	30 minutes	60 minutes
Number of patients (*n*)	23	16
Sex		
Male	15	11
Female	8	5
Age		
Median	57	53
Range	41–77	28–74
Performance status[a]		
0	12	4
1	6	6
2	5	3
Prior chemotherapy	19	15
Number of prior regimens		
0	4	1
1	5	9
2	8	2
⩾3	6	4
Prior radiation	8	10
Primary tumor		
Colorectal	8	8
NSCLC	2	0
Head and neck	1	0
Breast	2	1
Uterus and ovary	2	1
Other	8	6
Evaluable for response	14	8

[a] Not stated in three patients.

and on day 1 of 150-minute infusion schedule Venous blood samples were drawn before, 15 minutes after the beginning and immediately at the end of the S9788 infusion. Additional samples were drawn one hour, two, four, six, eight, 12, and 24 hours after the start of the S9788 infusion. The samples were rapidly centrifuged and the plasma frozen at −20 centigrade. The analysis was performed centrally using a HPLC assay [29]. Plasma levels were fitted to a two-exponential pharmacokinetic model. The area under the concentration time curve (AUC) was determined using the linear trapezoidal rule with extrapolation to infinity using the MicroPharm software.

Results

Patients characteristics

The patients' characteristics are depicted in Table 2. A total of 39 patients were enrolled in the study. The median age was 57 and 53 years for the first and second group, respectively. The median performance status was 1. Twenty-two patients had measurable lesions at study entry. In the first step of the study 23 patients received a total of 183 infusions (median six, range 2–21) of S9788 over 30 minutes alone or in association with doxorubicin (Table 1). In the second step, 16 patients were included receiving 87 infusions (median six, range 2–12) of S9788 over 150 minutes alone or with doxorubicin (Table 1). All patients suffered from advanced malignancy not amenable to curative treatment. The majority of the patients included were treated for metastatic colorectal cancer progressive after one or more prior chemotherapy regimens.

Table 3. Grade II + III non-cardiac toxicity.

	30-min Number of patients (%)	150-min Number of patients (%)	Total number of patients (%)
Neutropenia	1 (4)	3 (19)	4 (10)
Anemia	4 (17)	7 (44)	11 (29)
Nausea and vomiting	3 (13)	6 (38)	9 (23)
LFT-elevation	3 (13)	3 (19)	6 (15)
Injection site pain	8 (35)	3 (21)[a]	11 (30)
Asthenia	5	4	9 (23)
Somnolence	0	8 (50)	8 (21)

[a] Two patients with port-a-cath excluded.
Abbreviation: LFT – liver function tests (ASAT, ALAT, phosphatase).

Toxicities

The treatment was generally well tolerated by all patients (Table 3). No effects on the blood counts were detectable after the S9788 infusions alone. Following combined S9788 and doxorubicin infusions mild to moderate hematological toxicity was noted in a few patients only: grade II neutropenia in one of 23 patients with the 30-minute infusion, grade III neutropenia three of 16 patients receiving the 150-minute infusion. Grade II anemia (< 10 g/l) in a four of 23 and seven of 16 patients, respectively was attributable to the underlying malignant disease. Blood transfusions were not required. No thrombocytopenia was observed. Elevated liver function tests (⩾grade II) were found in six patients and are likely related to the primary disease. No elevation of the bilirubin was noted and no increase in the creatinine was observed. Grade III vomiting was observed in two patients receiving 72 mg/m^2 and 96 mg/m^2 of S9788 alone over 30 minutes in the absence of antiemetic prophylaxis. Nausea and vomiting were reported for 24 episodes in 11 patients receiving the 150-minute infusion, the intensity was not related to the S9788 dose.

Other toxicities scored as imputable to S9788 administration over 30 minutes were pain at the injection site in nine patients, tinnitus in two patients, altered color vision in two patients and thoracic pain in two patients. With the 150-minute infusion of S9788 pain at the injection site was reported in 34 cycles and in nine of the 14 patients treated by peripheral i.v. Further were noted somnolence in 11 patients (24 episodes) and malaise or vertigo during 11 infusions in five patients treated at doses ⩾96 mg/m^2.

Cardiac toxicity

30-Minute infusion of S9788

Pretreatment ECGs were available in all patients. Seven dose levels with a 30 minute-infusion were explored. QT

Table 4. Arrhythmia and symptoms associated with a 30-minute S9788 infusion.

Patient number	Dose level	Day	Holter records	Clinical symptoms	QTc (msec)	ΔQTc (%)
17	6	8	AV block I Bradycardia 50/min	Malaise	540	
18	7	1	Bradycardia 47/min		548	38
19	7	1	Bradycardia 48/min Junctional rhythm	Malaise	474	17
		8	Bradycardia 53/min Junctional rhythm	–	488	18
		22	Bradycardia 52/min Junctional rhythm	–	480	17
21	7	1	AV block I Bradycardia 50/min	Somnolence	518	30
22	7	8	AV block I	Malaise Nausea Somnolence	582	40
23	7	1	AV block II (Wenkebach) Bradycardia 35/min	Malaise Vomiting Anxiety	501	25

prolongation and arrhythmias were analyzed by Holter monitoring from DL 6 (72 mg/m^2) on. One patient treated at DL 6 (72 mg/m^2) and three of the six patients treated at DL 7 (96 mg/m^2) developed symptomatic cardiac arrhythmia (Table 4). Patients complained of vagal reaction, the systolic blood pressure temporarily dropped below 100 mmHg, and the Holter records showed QT-prolongation. Four patients developed bradycardia (35–53 beats/min.) during six drug-infusions. In three patients it was associated with a prolongation of the PQ-interval (two patients) or a second degree AV-block (type Wenckebach, one patient). One patient developed bradycardia and a junctional escape rhythm during three of the four S9788-infusions. All ECG-changes normalized rapidly after the end of the S9788 infusion. These conduction disturbances associated with symptoms in three of six patients at the DL 7 (96 mg/m^2) were considered dose limiting toxicity, and accrual to the 30 minutes schedule was stopped.

150-minute infusion of S9788

Baseline ECG was normal in all patients. A total of six dose levels with 87 infusions were explored. At dose level 9 (96 mg/m^2) one patient developed torsade de pointe, documented by Holter-monitoring. The patient remained asymptomatic. However, it was then decided, to de-escalate to 80 mg/m^2 and to subsequently reconsider dose escalation.

Asymptomatic arrhythmia were documented by Holter-monitoring in two of the sixteen patients treated with a 150-minute infusion of S9788. A torsade de pointe was observed at 96 mg/m^2 and a bigeminy in one patient at 104 mg/m^2. One patient treated at 144 mg/m^2 developed a third degree AV-block. None of these patients experienced any subjective complaints.

Table 5. QT-prolongation during the 150-minute S9788 administration.

Dose level (mg/m^2)	Number of patients	Mean QTc max (msec)/(range)	Mean ΔQTc (%)/(range)
72–80	5	491 (439–546)	24 (13–37)
96–104	6	526 (458–577)	28 (16–42)
120	3	494 (487–502)	23 (19–29)
144	2	562 (557–568)	42 (39–44)

In all but two patients, a decrease in the heart rate during the infusion was observed. Bradycardia (< 60 beats/min) was documented in six patients for 27 of 70 evaluable courses (39%). However the heart rate dropped below 50 beats/minute in only two patients.

Prolongation of the QT-interval was documented at all dose levels. At doses of ⩾ 120 mg/m^2 QT-prolongation was present in all patients (Table 5). Large inter-individual variations of QT-prolongation were observed and a dose 144 mg/m^2 S9788 was considered the MTD since both patients had QTc values > 550 msec corresponding to a 39% and 44% increase of the basal values. This condition known to favor torsade de pointe or ventricular arrhythmias was considered the dose limiting toxicity and the study was closed.

We specifically analyzed whether the patients experiencing cardiac arrhythmias had a history of cardiovascular disease or electrolyte laboratory abnormalities. None of these patients had previously known cardiac disease or hypertension. Baseline ECG was normal in all patients. Of the patients experiencing cardiac dysfunction, four patients (two patients in each study) had grade 1 hypokalemia. The other electrolytes (sodium, potassium, chloride, calcium, phorphorus) were within normal limits. The patient developing a torsade de pointe has been treated with hydrochlorthiazide/amiloride for over a year because of peripheral edema. No other patient received vasoactive drugs or drugs known to prolong the QT-interval.

Reversibility of QTc prolongation

Prolongation of the QT-interval (> 440 msec) was observed at all dose levels. When corrected for the heart rate, no definitive correlation between dose level and QTc-interval could be demonstrated. The QTc was analyzed in 76 Holter-ECG 60 minutes after starting S9788. The median QTc-value was 494 msec, and the mean 505. QTc values over 600 msec were measured in one patient at dose level 6 (72 mg/m^2) and 7 (96 mg/m^2) of the 30-minute infusion, and in one patient at dose level 9 (96 mg/m^2 over a 150-minute infusion). All observed arrhythmias and ECG changes were fully reversible and normalized usually within less then 12 hours after discontinuing the S9788 infusion.

Torsade de pointe

In one patient asymptomatic torsade de pointe occurred during the second week of treatment, whose case is described in detail:

This 64 year old obese woman was diagnosed five years earlier with adenocarcinoma of the rectum, stage Dukes C. She was initially treated by surgery and adjuvant radiotherapy and an isolated liver metastasis was resected 11 months later. A second resection of a liver metastasis and a loco-regional recurrence was performed four years after diagnosis, followed by chemotherapy with 5-FU and leucovorin. The patient progressed after 6 cycles of chemotherapy and enrollment in this trial with S9788 and doxorubicin was suggested. Her past medical history was unremarkable; for mild-moderate peripheral edema she has been taking daily hydrochlorthiazide 50 mg/amiloride 2.5 mg for over one year. Baseline cardiac evaluation by echocardiography showed a normal left ventricular function without signs of hypertrophy or dilatation. Laboratory evaluation was unremarkable with a sodium 138 mmol/l, a potassium 3.5 mmol/l, a calcium of 2.28 mmol/l and phosphor of 0.8 mmol/l. Magnesium was not done. The ECG showed an unremarkable sinus rhythm with normal QT-interval. A total dose of 170 mg S9788 (DL 9: 96 mg/m^2) was administered over 150 minutes on day 1, and on day 8 in association with doxorubicin (20 mg). At the end of S9788 administration on day 1 QT-prolongation was documented by ECG. The QTc-interval before the first S9788 administration was 430 msec, at the end of the infusion 470 msec, Holter monitoring revealed rapid normalization of the QT-interval and no significant arrhythmias. On day 8, baseline QTc-interval was 438 msec (Figure 1a), at the end of the infusion 590 msec (Figure 1b). Ten minutes after the end of the S9788 perfusion (about two hours after doxorubicin administration), an isolated episode (duration < 10 seconds) of torsade de pointe was registered on Holter-monitoring (Figure 1c). The patient remained asymptomatic. Electrolytes drawn before the infusion were unremarkable with a sodium of 139 mmol/l, potassium 3.7 mmol/l, chloride 100 mmol/l, calcium 2.49 mmol/l and phosphor 1.1 mmol/l. On follow-up the Holter ECG and subsequent ECG's were again normal. The patient died of disease progression three months later.

Maximum tolerated dose

With both administration schedules arrhythmia was dose limiting. Both, on ECG and Holter monitoring, QT-prolongation, ventricular tachycardia and torsade de pointe were the predominant findings. With the shorter infusion schedule patients experienced malaise and temporarily blood pressure drop, The protracted S9788 infusion over 150 minutes induced ventricular tachycardia and torsade de pointe without any noticeable symptoms.

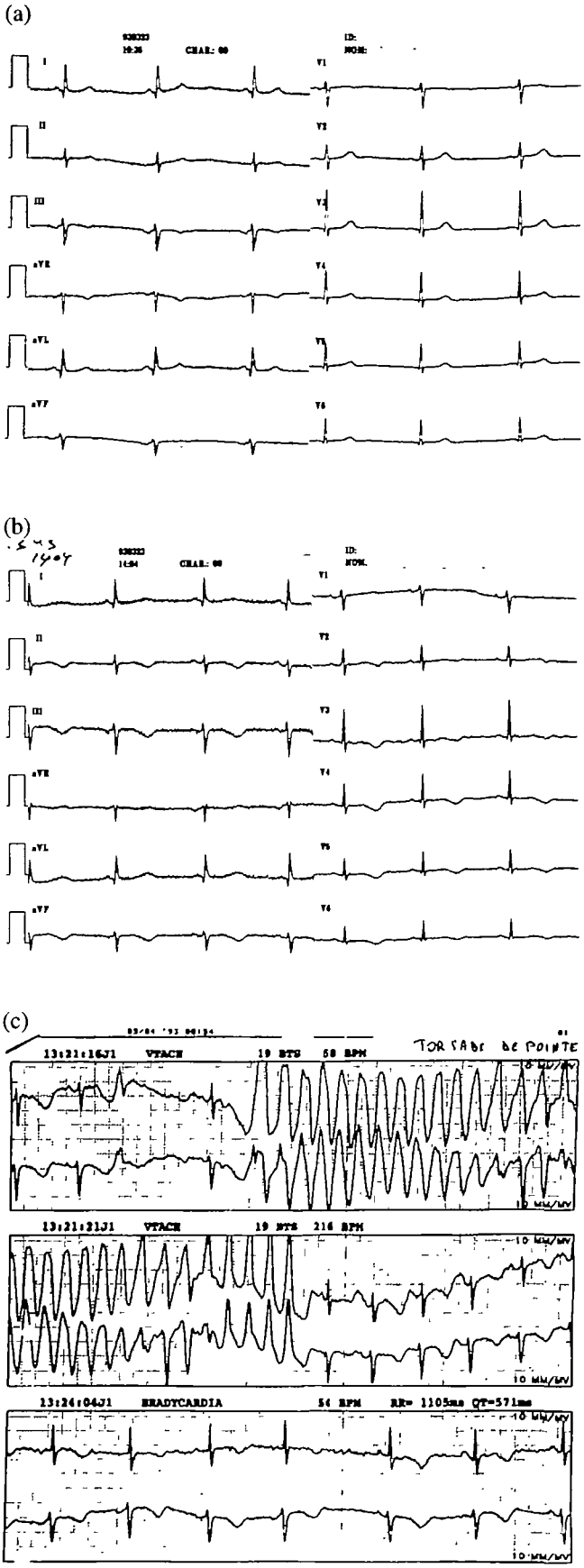

Figure 1. EKG changes and torsade de pointes during S9788 30-minute infusion. (a) EKG before S9788: heart rate 50/min, QTc-time 438 msec. (b) EKG at the end of S9788 infusion: heart rate 54/min, QTc-time 590 msec. (c) Holter during S9788: torsade de pointes initiated by a short–long–short sequence.

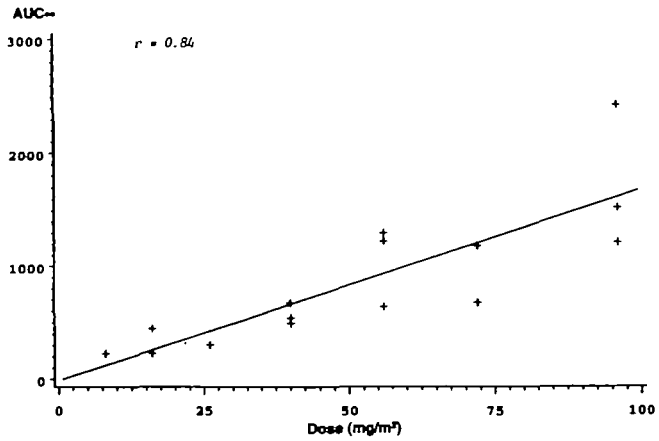

Figure 2. AUC∞ *versus* dose. S9788 30-minute infusion: AUC extrapolated to infinity (μM × h) *versus* dose level (mg/m^2) during cycle 1.

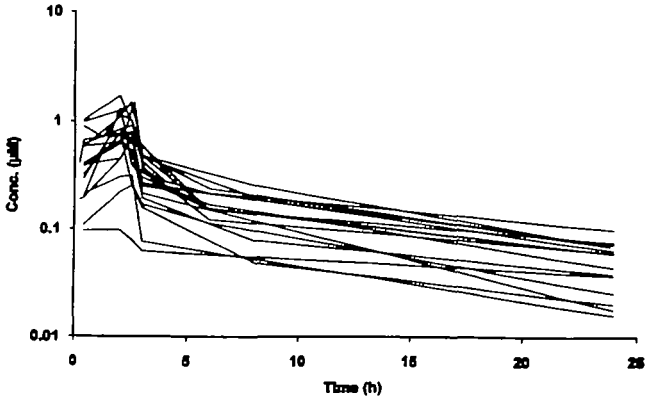

Figure 3. Plasma concentrations S9788. S9788 150-minute infusion: plasma concentrations (μM) after escalating doses of S9788 during cycle 1.

We consider a dose of 96 mg/m^2 of S9788 as the MTD for a 30 minute infusion schedule, and 144 mg/m^2 for a 150 minutes infusion duration, the recommended doses for phase II evaluation are 72 mg/m^2 as a 30-minute infusion and 104 mg/m^2 as a 150-minute infusion.

Responses

Twenty-two patients were evaluable for response. One partial remission in the lung was achieved with the 30-minute infusion schedule in a woman with breast cancer with bone and pulmonary metastases. This patient had previously received hormonotherapy. Immediately prior to inclusion in this trial she had progressed after two cycles of losoxantrone (DuP 941) treatment. No objective responses were observed in the other patients.

Pharmacokinetics

The results of the pharmacokinetic studies are here briefly summarized. [30, 31] In 16 patients receiving a 30-minute infusion of S9788 pharmacokinetic sampling was performed on day 1 (n = 16) and day 8 (n = 14). Maximal plasma levels measured ranged from 0.05–4.85 μM and linearly correlated with the dose administered. Plasma levels >0.5 μM were achieved at a doses >26 mg/m^2. A linear correlation was also demonstrated for the AUC *versus* the administered dose level (r = 0.84, Figure 2). Fitted to the bicompartment model the estimated half life alpha was 11.3 minutes (± 12.8), and the terminal half life of 13.5 hours (± 11.8). Plasma levels were available for all 16 patients on day 1 treated with a 150-minute infusion. Peak concentrations were achieved after 120–150 minutes and ranged from 0.1 μM – 1.5 μM with a large intersubject variability (Figure 3). Mean alpha half live was 13.2 minutes (±8.4), and terminal half live 15.0 hours (±9.6). In eight patients the plasma concentrations of S9788 could be correlated to the QTc-time (Figure 4). The QTc prolongation duration appeared to be dose-dependent (Figure 5).

Discussion

Intrinsic and acquired multidrug resistance remains a major impediment for successful treatment with anticancer agents. One approach to overcome MDR is the modulation of Pgp. Several drugs have been recognized *in vitro* and *in vivo* to interfere with Pgp-function. Calcium channel blockers like verapamil are able to reverse MDR by competitive inhibition of Pgp. However, at the clinically effective doses, cardiovascular toxicity with hypotension and congestive heart failure limits their clinical applicability [12]. Steroid derivatives like tamoxifen and megestrol are also known for their ability to revert multiple drug resistance. In two trials with vinblastine high doses of tamoxifen (up to 150 mg/m^2 twice daily) have been administered. Observed toxicities, although not considered dose limiting, consisted of tremor, hyperreflexia, dizziness and asymptomatic QT-prolongation on ECG [32, 33]. Cyclosporine A has also been extensively investigated in the clinic for Pgp-modulation [34–36]. With a 60-hour continuous infusion a clinical effect could be demonstrated with increased etoposide [35, 36] and doxorubicin [34] exposure and increased toxicity, mainly myelosuppression, nausea and vomiting and hyperbilirubinemia [35, 36]. Currently, a new and nonimmunosuppressive cyclosporine analogue is undergoing clinical testing [17]. In a phase I trial in association with etoposide severe ataxia was the DLT and other common toxicities were paraesthesiae, hyperbilirubinemia and myelosuppression [17].

In order to specifically reverse MDR and modulate Pgp the triazinoaminopiperidine derivative S9788 was developed by Servier Research Laboratories. *In vitro* and *in vivo* preclinical models showed promising activity in reversing the Pgp-function with very little toxicity. *In vitro*, S9788 was more active than verapamil and showed similar activity with cyclosporine. In the mouse model DLT was neurological with clonic seizures. Beagle dogs presented with moderate ataxia and seizures at high and very high doses (7.2 mg/kg and 14.5 mg/kg, respectively). After the observation of conduction abnormalities during the phase I study, new *in vivo* testing in

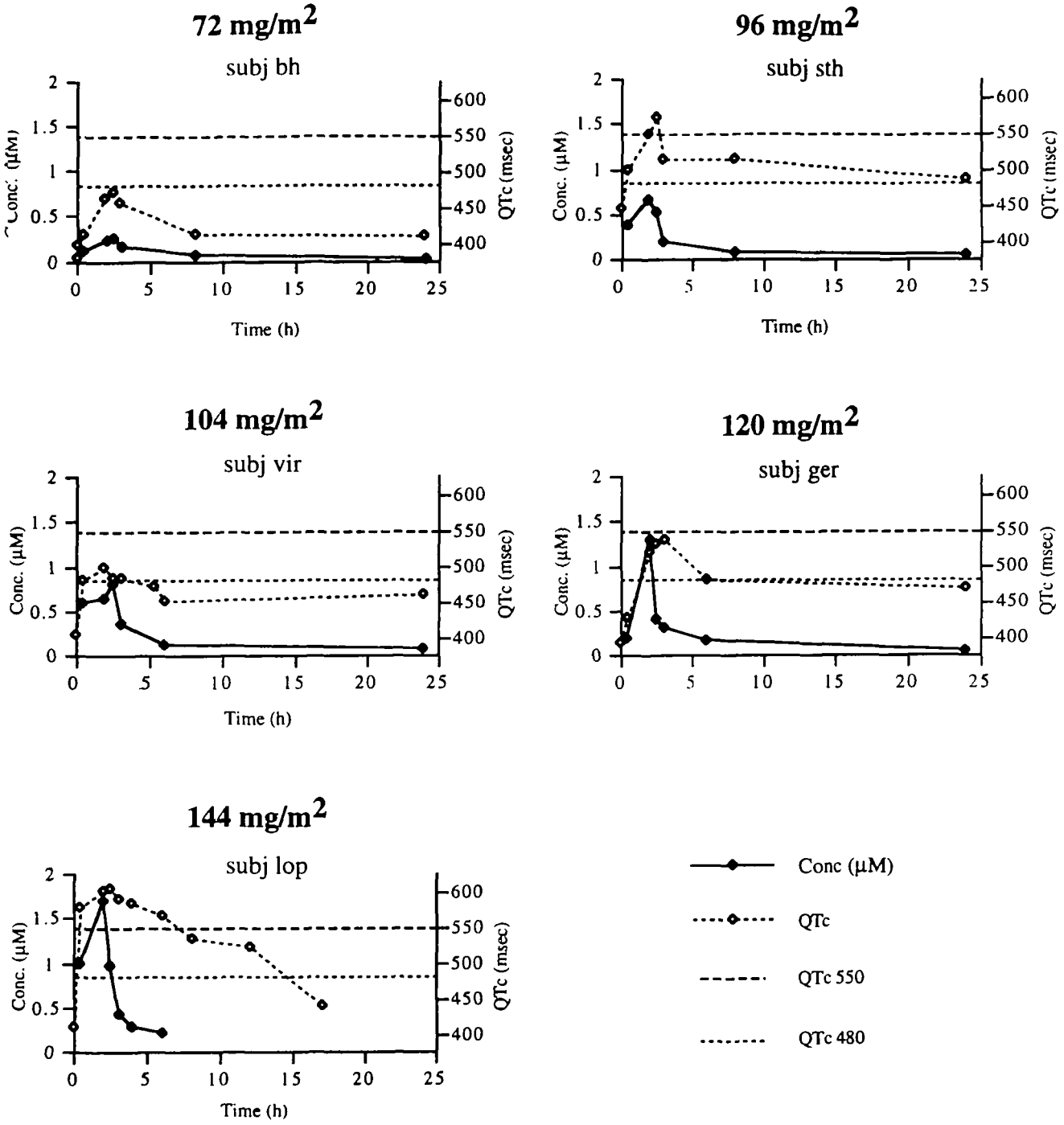

Figure 4. Plasma concentrations and QTc-prolongation. S9788 150-minute infusion: plasma concentrations and QTc-prolongation *versus* time in individual patients at different dose levels.

anesthetized dogs was performed. Administration of S9788 at 10 mg/kg bradycardia, conduction blocks and prolongation of the QT-interval were demonstrated. Similarly to verapamil S9788 may inhibit the calcium channels, although its affinity is 20 times lower than verapamil. However, in a model with isolated rat Purkinje fibers S9788 also inhibits rapid sodium influx and thus diminishes the contractility of the myocardium. This sodium channel block is possibly the cause for the conduction abnormalities and the susceptibility to arrhythmia observed.

We evaluated toxicity of weekly S9788 administration on two different administration schedules. Dose limiting toxicity was reached with a 30-minute administration duration at 96 mg/m^2, and at 144 mg/m^2 when S9788 was infused over 150 minutes. With both administration schedules DLTs were cardiac arrhythmia with QT-prolongation, AV conduction blocks, premature beats and torsade de pointe. Symptomatic arrhythmia were only observed with the shorter infusion schedule. Vagal type malaise were reported in three of six patients treated at the highest DL (96 mg/m^2) of the 30-minute infusion.

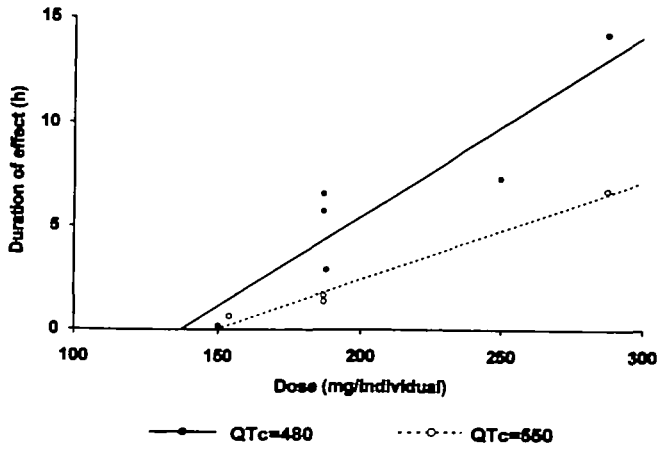

Figure 5 QTc-prolongation *versus* dose. S9788 150-minute infusion: duration of QTc-prolongation over 480 and 550 msec, respectively in function of the total dose of S9788 administered.

With the 150-minute infusion schedule QT-prolongation, bigeminy and torsade de pointe were demonstrated on ECG and Holter-24-hour cardiac monitoring, but none of the patients was symptomatic. While carefully analyzing the data, no correlation of cardiac susceptibility, prior cardiovascular disease, concomitant vasoactive medication or electrolyte disturbances was evident. All observed cardiac abnormalities were rapidly reversible after S9788 discontinuation.

Torsade de pointe describes a syndrome of ventricular tachycardia with polymorphic oscillations in the QRS-height in association with lengthening of the QT-interval of usually more than 500 msec [37]. The tachycardia occurs due to early afterdepolarizations and may result in ventricular fibrillation. Hypokalemia and hypomagnesemia may be a predisposing factors. Prolongation of the QT-interval has also been recognized as a rare familial disorder frequently leading to ventricular arrhythmias and sudden death [38, 39]. Acquired QT-prolongation has been described in association with several drugs, like class IA and class III antiarrhythmics (quinidine, procainamide, disopyramide, sotalol, amiodarone), phenothiazines, tricyclic antidepressants and nonsedating antihistamines such as terfenadine [40]. In the current trial we observed one episode of torsade de pointe and ventricular tachycardia during the second S9788 administration over 150 minutes in a 64-year-old patient with no previous history of cardiac disease. On the day developing torsade de pointe, the ECG before the infusion showed sinus bradycardia (50 bpm), but electrolytes within normal limits on laboratory evaluation.

In two other phase I trials of S9788 with infusion schedules of 30 minutes and 30 + 150 minutes, QT-prolongation, AV conduction blocks, ventricular arrhythmias with one episode of torsade de pointe were again determined as the dose limiting toxicities [27, 41]. In a similar phase I study with a protracted S9788 administration over six hours doses up to 480 mg of S9788 could be administered. Again QT prolongation was present at all dose levels. QTc prolongation > 550 msec was documented during 25% and 29% of courses respectively at a S9788 dose of 240 and 320 mg, and in 67% and 41% of courses, at dose levels of 400 and 480 mg, respectively (Data on file, IRIS, Servier SA, Courbevoie, France). We treated six patients with a six-hour infusion for a total of 17 cycles at doses of 160, 240 and 320 mg/m^2 of S9788 with doxorubicin administration three hours after the S9788 infusion started. Significant QT-prolongation occurred at all doses. This trial prematurely closed after we observed another episode of torsade de pointe at a dose of 240 mg. Ventricular arrhythmias and one torsade de pointe were also reported in a French trial using a six-hour infusion [42]. A dose-effect relationship could not be clearly demonstrated [42]. The pooled data of over 230 patients treated with S9788 on different infusion schedules show a variable individual susceptibility, with QT prolongation observed at all dose levels and with all schedules. A correlation of the administered dose of S9788 and the extent of QTc-prolongation is suggested. Despite improved subjective tolerability of S9788 with a prolonged administration schedule, the risk of severe arrhythmias persisted with longer infusion durations. The *in vivo* measured concentrations correspond to the *in vitro* determined concentrations necessary for reversing MDR (0.5–5 μM). The short first half life may make a longer infusion duration necessary for a clinically significant MDR reversal [8]. In order to limit QTc prolongation in the majority of patients to < 550 msec, the plasma concentrations of S9788 should not exceed 0.4 μM. For a QTc time < 480 msec our model suggests concentrations ≤0.3 μM. This contrasts with the required minimally effective *in vitro* S9788 concentration which is estimated at 0.5 μM [43]. Based on these considerations, it was decided not to pursue the further development of S9788.

Acute cardiac toxicity is unusual in daily oncology practice. Unlike anthracycline induced cardiomyopathy or congestive heart failure, S9788 induced conduction and repolarization disturbances may be considered as potentially life threatening adverse events. Only prospective and systematic evaluation for this type of adverse event is able to detect potentially dangerous changes. The observed prolongation of the QT-interval with S9788 predisposes to ventricular arrhythmia and torsade de pointe. In our trial, the occurrence of severe arrhythmia due to the QT prolongation, modulated by individual susceptibility, underlying unrecognized cardiac disease, electrolyte imbalances and other unidentified parameters were considered too unpredictable and potentially dangerous. The low benefit/risk ratio did not justify further clinical development of S9788.

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